

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Applicants: Henry Daniell

Examiner: Ann Kubelik

Serial No.: 10/519,821

Art Unit: 1638

Filed: 9/28/2005

Confirmation No. 3102

For: PLASTID GENETIC ENGINEERING
VIA SOMATIC EMBRYOGENESIS

DECLARATION OF HENRY DANIELL, PhD.

I, Henry Daniell, Ph.D. hereby declare and say as follows:

THAT, I am employed as a professor at University of Central Florida, Orlando, FL.;

THAT, I am the above-named Applicant and inventor of the subject matter described and claimed in the above-identified patent application;

THAT, by virtue of my educational and employment background, my attendance at seminars, my ongoing research, my continuing review of scientific periodicals and journals, and through correspondence with professional colleagues, I am aware of the level of skill of one ordinarily skilled in the art of plant genetics, and in particular, chloroplast transformation;

THAT, I have studied the application Serial No. 10/519,821 and office actions which have been issued during prosecution of this application (including cited references), as well as responses which have been filed on the Applicants' behalf, and being thus duly qualified declare as follows:

1. The Patent Office has rejected pending in view of WO 99/10513, Daniell publication. I am an inventor on said Daniell Publication. The Daniell publication represents a substantial piece of work relating to my development of vectors designed for

chloroplast transformation and methods of using such vectors, for chloroplast transformation, among other things. However, the Daniell publication does not teach or enable methods of chloroplast transformation and regeneration through somatic embryogenesis. This quite simply had not been achieved nor developed by myself at that time or, to my knowledge, by anyone else.

2. Indeed, the work set forth in the subject application, is to my knowledge, the first demonstration of chloroplast transformation and regeneration of homoplasmic plants achieved through somatic embryogenesis. It is fair to say that this is an important and remarkable achievement in the art. Not all plants are capable of regeneration through organogenesis, thus, before the teachings of the subject application, many important plant species were incapable of producing plants that had uniform and stable chloroplast transformation.
3. By way of background and context illustrating the importance of the teachings of the subject application, one must realize that each plant cell contains up to 10,000 copies of chloroplast genomes. In order to achieve successful chloroplast transformation, all 10,000 copies should have integrated foreign genes (homoplasmy) and there should not be a mixture of transformed and untransformed chloroplast genomes (heteroplasmy). Homoplasmy has been so far achieved in crops that produce shoots directly from leaves bombarded with foreign genes. When plants produce shoots directly from leaves, this process is described as organogenesis. When heteroplasmic condition is observed, these shoots are cut into small pieces and regenerated again under stringent selection conditions to eliminate untransformed chloroplasts or wild type chloroplast genomes. After several rounds of selection, homoplasmic shoots are obtained.
4. However, several plant species (many monocot species in particular) do not regenerate via organogenesis but each embryogenic cell forms an embryo. This is called a somatic (vegetative) embryo to distinguish it from embryos that are usually formed after sexual reproduction. The somatic embryo then gives rise to the shoot and root. It is not possible to chop embryos to small pieces and regenerate shoots to

achieve homoplasmy. Although chloroplast transformation was achieved via organogenesis in 1990, until the invention set forth in the present application, chloroplast transformation via somatic embryogenesis has been elusive and unsuccessful in several laboratories around the world.

5. In developing the somatic embryogenesis process, I identified several challenges and developed solutions to these challenges in order to accomplish chloroplast transformation via somatic embryogenesis. Some of these included identifying appropriate regulatory sequences and selectable marker(s) that function in non-green and green chloroplasts, and being able to regenerate chloroplast transgenic plants via somatic embryogenesis and achieve homoplasmy, which lacks the benefit of subsequent rounds of regeneration offered by organogenesis. Understanding and manipulating the somatic embryogenesis system, which lacks the advantage of subsequent rounds of regeneration from heteroplasmic tissues, added to the difficulty and complexity of this endeavor.
6. I realized that during transformation, transformed non-green plastids must develop into mature chloroplasts and transformed cells will need to survive the selection process during all stages of development. Therefore, another major challenge was to discover how to provide plastids an ability to survive selection in the light and the dark, at different developmental stages. This was absolutely critical because I also recognized that only one or two chloroplasts are transformed in a plant cell after bombardment and that these plastids will need to have the ability to survive the selection pressure, multiply and establish themselves while all other untransformed plastids are eliminated in the selection process. Therefore, leading to the ultimate goal of achieving chloroplast transformation in plants that regenerate via somatic embryogenesis, I had to develop and create suitable chloroplast vectors, had to properly deliver DNA into competent recipient cells and ensure integration into one or two chloroplast genomes (heteroplasmy). Further, I had to devise a system that was capable of replacing all native (wild type) chloroplast genomes with transformed chloroplast genomes to achieve homoplasmy, inducing somatic embryos from homoplasmic embryogenic cells and regenerating transgenic plants. Through

substantial effort and expertise, I was able to resolve each of these identified requirements. The result of this substantial effort is set forth in the subject application. Thus, in my expert opinion, the subject application represents, for the FIRST time, a complete and enabled process to achieve chloroplast transformation in plants that regenerate via somatic embryogenesis.

7. Though the work disclosed in the Daniell publication represents a remarkable achievement in its own right, the publication does not contemplate the particular hurdles outlined above, nor solutions and method of overcoming those hurdles. Thus, in my opinion, the cited Daniell publication does not provide the requisite teaching that would enable one skilled in the art to achieve chloroplast transformation through somatic embryogenesis, without undue experimentation.
8. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information in belief are believed to be true; and further that these statements were made with the knowledge that willful false statements in the like so made are punishable by fine or imprisonment, or both, under ' 1001 of title 18 of the U.S.C. and that such willful false statements made jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.



Henry Daniell, Ph.D.

June 6, 2010

Date